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Hypervariable Region Diversity of Hepatitis C Virus and Humoral Response: Comparison Between Patients With or Without Cirrhosis

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To investigate the potential clinical utility of antibody response to HVR1 of HCV, the genomic and amino acid diversity of HVR1 was compared between two groups of four chronic HCV carriers with or without liver cirrhosis. Peptides corresponding to the deduced COOH- and NH2-terminal amino acid sequences of HVR1 were synthesised to assess the reactivity of patient sera to autologous and homologous HVR1 epitopes by enzyme-linked immunosorbent assay. HCV chronic carriers had significantly more frequent cross-reactivity with homologous C- than N-terminal HVR1 peptides. Twelve cirrhotic and eleven noncirrhotic patients had a similar frequency of cross-reactivity with either C- or N-terminal HVR1 peptides. However, noncirrhotic patients had a significantly higher level of C-terminal HVR1 antibody cross-reactivity than cirrhotic patients. In HCV chronic carriers, the magnitude of the immune response to but not the frequency of cross-reactivity with C-terminus HVR1 peptides differ between patients with and without liver cirrhosis. ***J. Med. Virol. 59:25–31, 1999.*** © 1999 Wiley-Liss, Inc.

INTRODUCTION

Most individuals infected with hepatitis C virus (HCV) remain viraemic for many years before the onset of clinically apparent liver disease. Clinical progression is not related directly to virological factors such as the extent of viraemia or the presence of specific viral subtypes [Tanaguchi et al., 1993]. HCV circulates as a heterogeneous population of related variants that constitute quasispecies. The observed viral genomic diversity probably results from an inability of this RNA virus to proofread during transcription, and it may play a role in viral persistence as a result of the constant generation of variants capable of escaping host immune surveillance [Tagariello et al., 1995]. Hypervariable region 1 (HVR1), located close to the NH2-terminus of the main envelope protein E2, has been studied at the nucleic acid level in serial samples from HCV carriers and in groups of patients with various histological stages of liver disease by single-strand conformation polymorphism (SSCP) analysis or nucleotide sequencing [Enomoto et al., 1994; Honda et al., 1994; Koizumi et al. 1995; Moribe et al. 1995; Toyoda et al. 1996]. Such studies suggested that progressive liver disease is correlated with increased genomic diversity of HVR1 rather than with duration of infection [Honda et al., 1994; Moribe et al., 1995; Toyoda et al., 1996].

In contrast, van Doorn et al. [1994, 1995] showed that in chimpanzees infected with identical viral isolates, a high level of HVR1 genomic diversity was apparent in animals who recovered but relative homogeneity was observed in the animal who progressed rapidly. The researchers suggested that genomic diversity is driven by selective immune pressure against the E2 hypervariable region. We have presented preliminary HVR1 sequence analysis from seven patients either with mild liver disease or undergoing liver transplantation for end-stage cirrhosis. Our results suggested that, in transplanted patients, genomic diversity was reduced before and further reduced after transplantation, consistent with the hypothesis that high genomic diversity reflects strong immune selective pressure and corresponds to a favourable clinical prognosis [Lawal et al., 1997].

Evidence suggests that HVR1 may mediate the binding of HCV to susceptible cells [Zibert et al. 1995; Rosa et al., 1996] and may also act as a site of neutralisation [Farci et al., 1996; Rosa et al., 1996]. HVR1 is immunogenic, containing two distinct epitopes located between amino acids 11 and 27 [Kato et al., 1993]. Because the amino acid sequence in this portion of HVR1

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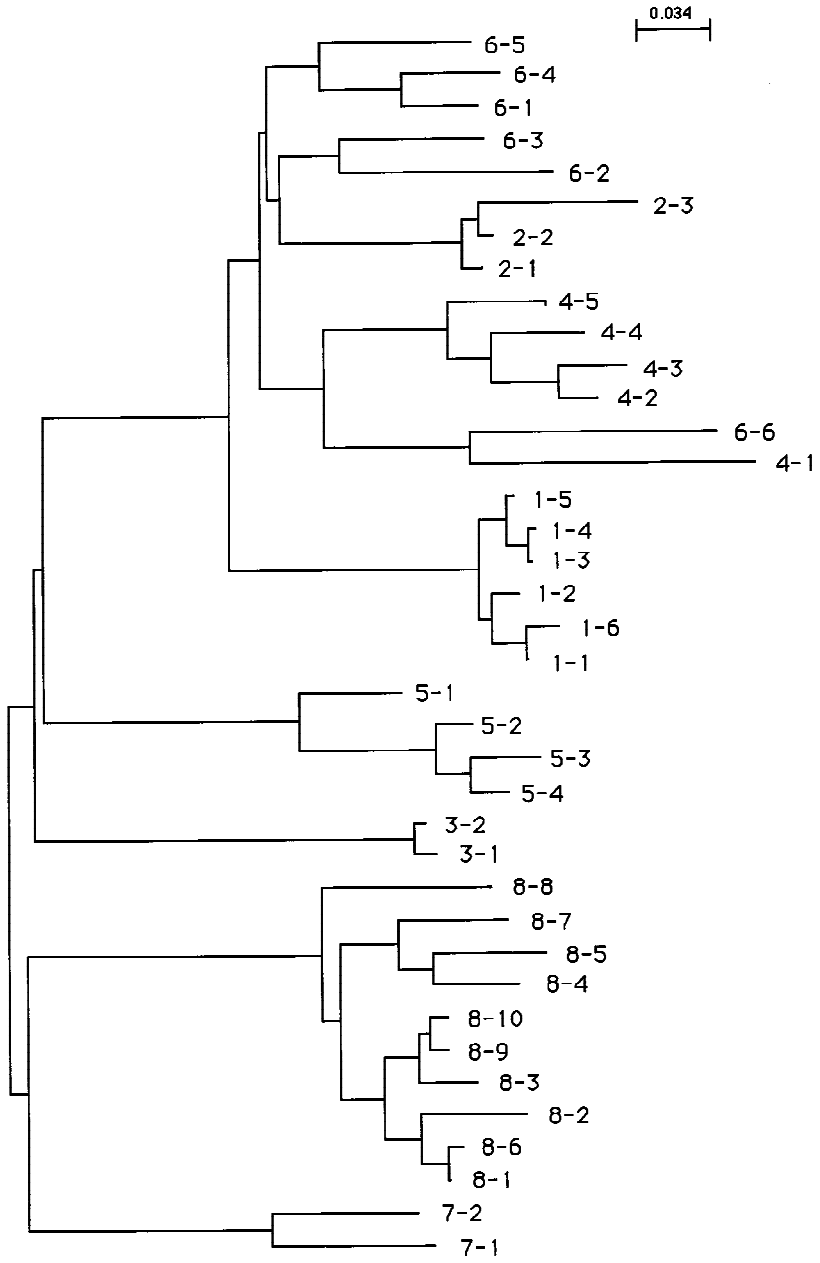

Fig. 1. Unrooted phylogenetic tree of the aligned sequences of nucleotides 1462 to 1620 of HCV, spanning the 3′-end of E1 and the 5′-end of E2 envelope proteins including HVR1. The sequences were aligned using the Clustal W multiple alignment programme and the tree was constructed from the distance matrix based on the pairwise comparison of all sequences using the Neighbor Joining method [Saitou and Nei, 1987], resulting in a tree with the minimal length of all branches. The length of each branch is proportional to the percentage of divergence between sequences. The scale at the top right corner of the figure indicates the percentage of divergence per length.

is highly divergent between patients, it was initially thought that these epitopes elicit highly specific antibodies. However, patient sera exhibit substantial cross-reactivity even with HVR1 peptides that share less than 50% sequence identity with the corresponding endogenous HVR1 sequences [Scarcelli et al., 1995; Zibert et al., 1995; Jackson et al., 1997]. On the basis of these data, it was hypothesised that the magnitude and breadth of the antibody response to HVR1 may play an important role in the ability of the host to restrict viral replication and, thereby, limit progressive liver disease [Lawal et al., 1997].

To test this hypothesis, HVR1 genomic variability and the corresponding immune response to autologous HVR1 peptides were compared between four patients with end-stage cirrhosis and four individuals who have remained HCV carriers for more than 12 years without cirrhosis. Cross-reactivity with these peptides was also tested with sera from HCV chronic carriers with or without severe liver disease.

TABLE I. HVR1 Peptides Identified From Study Subjects and Tested in ELISA

Peptides[a]	Sequence (single letter code)[b]															
1-1		T	Q	H	V	T	S	I	F	S	F	**G**	S	S	**Q**	N	
1-4		—	—	—	—	—	—	L	—	—	—	—	—	—	—	—	
2-1	T	T	S	G	F	A	S	L	F	K	F	**G**	P	S	**Q**	H	
2-3		—	—	—	—	—	—	—	—	R	L	—	—	—	—	—	
3-1	T	T	S	G	L	A	G	L	F	N	S	**G**	A	R	**Q**	H	
3-2		—	R	—	—	—	—	—	—	T	—	—	—	—	—	—	
4-1	N	T	H	G	I	A	S	L	F	A	F	**G**	P	A	**Q**	K	
5-1	N	T	Y	G	L	T	A	L	L	T	R	**G**	P	S	**Q**	Q	
5-4	I	V	N	R	F	—	S	F	F	N	L	—	—	—	—	R	
6-1	D	T	A	G	L	A	G	L	F	N	L	**G**	P	K	**Q**	T	
6-2	A	—	S	A	—	V	—	—	L	S	P	—	A	—	—	N	
7-1			G	G	V	A	G	L	F	K	M	**G**	S	Q	**Q**	K	L
7-2			—	M	F	T	—	—	—	N	Q	—	A	—	—	—	—
8-1			N	R	L	G	G	L	F	N	F	**G**	P	K	**Q**	K	I
8-2			K	G	—	N	—	—	—	D	L	—	—	—	—	—	—
8-3			Y	G	—	T	T	—	—	—	P	—	—	R	—	Q	—
8-4			S	G	—	S	S	—	—	T	P	—	—	—	—	Q	—
8-8			—	—	F	A	—	M	—	S	L	—	A	R	—	—	—

[a]The first digit indicates the patient number, the second indicates the HCV variant number.
[b]Sequence identity with the 1st peptide from each patient is indicated by a dash. The G and Q residues in bold type are common to all sequences.

Patients and Methods

Eight patients infected with HCV were included in the study. All were positive for antibodies to HCV according to standard criteria for antibody screening and confirmation testing with RIBA3 (Ortho Diagnostics, Raritan NJ). HCV RNA was detected in all patients by nested polymerase chain reaction analysis [Lawal et al., 1997]; the extent of viraemia ranged from $<1 \times 10^3$ to 1.5×10^7 genome equivalents/mL. HCV was subtype 1b from patients 1, 2, 4, and 6, subtype 1a from patients 3, 5, and 7, and subtype 2b from patient 8. Typing was based on sequencing of the E1–E2 region according to Okamoto et al. [1992].

Patients 1 to 4, one woman and three men, ranged in age from 46 to 66 years, had end-stage liver cirrhosis, and were admitted for liver transplantation. Patients 5 and 6 were blood donors detected by routine anti-HCV screening who had been intravenous drug users in the 1970s. In 1994, patient 5, a 46-year-old man, had serum alanine transferase (ALT) values that ranged between normal and three times the upper normal limit, as well as stage 1 fibrosis [Wong et al., 1996]. Patient 6, a 37-year-old woman, showed persistently normal ALT values and stage 2 fibrosis. Patient 7, a 45-year-old haemophiliac with a high titre of inhibitor to factor VIII but negative for antibodies to human immunodeficiency virus (HIV), was shown to be positive for anti-HCV in 1991. His serum ALT values were consistently less than three times the normal upper limit; clinical examination and ultrasound analysis were normal. Patient 8, a 28-year- old man with severe haemophilia A, was positive for antibodies to factor VIII but negative for anti-HIV. He also had no clinical signs of chronic liver disease and his ultrasound examination was normal. In the two patients with haemophilia, the presence of an antibody to factor VIII prevented the performance of a liver biopsy. On the basis of the clinical data and liver histology where available, patients 5 to 8 were considered noncirrhotic chronic carriers of HCV, having been infected for at least 12 years.

Genomic sequencing of HCV in the E1–E2 region, including HVR1 was performed as previously described [Lawal et al., 1997] with serum specimens obtained from patients 1 to 3 before transplantation, from patient 4, 31 days after transplantation (considered representative of the pretransplant viral population), and from the untreated, noncirrhotic patients 5 to 8. At least 10 cDNA clones were sequenced in order to identify mutations in HVR1 and to estimate the number of variants in each specimen. An unrooted phylogenetic tree was constructed from the multiple alignment of 158 nucleotides (nt) encompassing HVR1 [nt 1462 to 1620 according to Choo et al., 1991], with the use of the Clustal W program [Higgins et al., 1988].

Two sets of synthetic C- and N-terminal HVR1 peptides were prepared. The first set consisted of the 15 or 16 COOH-terminal amino acids of HVR1 deduced from dominant and minor variants containing replacement mutations from each patient. The second set corresponded to the NH2 terminal 15 amino acids of the same HVR1 sequences; since this part of HVR1 was considerably less variable than the C-terminal part, some patients had identical NH2-terminal and different COOH-terminal peptides. Peptides were modified by the addition of a cysteine residue at the NH2-terminus, cross-linked through this residue to bovine serum albumin, and used as antigens to coat microtitre plates for the detection of anti-HVR1 in autologous sera and cross-reactivity with sera from all other patients as described previously [Jackson et al., 1997].

TABLE II. Antibody Reactivity Against C-Terminal HVR1 Peptides in Patients With and Without Cirrhosis

Peptides[a] HCV sera	1-1	1-2	2-1	2-3	3-1	3-2	4-1	5-1	5-4	6-1	6-2	7-1	7-2	8-1	8-2	8-3	8-4	8-5
Cirrhotic																		
No1	**1.9**[b]	**2.0**[c]	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
No2	—	—	**2.0**	**—**	7.2	4.4	12.6	—	1.4	7.2	—	—	6.9	—	1.0	1.1	1.5	—
No3	—	—	—	1.4	**6.3**	**3.3**	—	—	—	1.3	—	—	—	—	—	1.0	1.5	6.2
No4	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1.0	—	—
B304	—	0.9	1.8	1.1	2.7	7.5	32.1	1.0	1.0	1.2	1.1	1.7	—	—	—	—	1.0	1.5
D202	—	1.3	2.4	1.6	4.9	—	2.2	1.7	—	—	—	1.0	1.2	—	—	—	—	—
J403	—	1.2	—	1.6	—	—	1.5	—	—	1.7	—	—	—	1.7	—	—	2.4	1.3
L305	—	1.1	1.0	—	5.4	8.0	1.6	—	1.0	1.0	—	4.9	—	—	—	—	1.0	1.2
M1003	—	6.1	—	—	—	—	2.4	—	—	—	—	3.0	18.5	—	—	—	—	4.5
M2501	—	1.1	2.3	1.5	—	1.5	5.6	2.3	9.0	1.7	—	4.6	—	—	2.2	2.1	—	—
M606	—	—	—	—	—	1.6	1.0	—	—	—	—	—	—	—	—	—	2.6	—
W801	—	—	1.1	—	—	—	—	1.2	—	—	—	—	1.1	1.1	1.1	1.0	1.1	—
Non-cirrhotic																		
No5	—	—	—	—	6.8	4.3	12.0	**2.8**	**8.4**	1.3	—	1.2	—	9.2	14.5	3.4	18.9	—
No6	—	—	—	—	3.8	2.0	—	—	—	**3.2**	**14.3**	—	—	—	2.4	13.0	12.7	2.9
No7	—	2.1	7.3	8.4	3.9	4.3	2.4	—	—	—	—	**14.7**	**1.5**	1.5	3.3	5.6	14.3	6.4
No8	—	—	1.7	2.2	8.3	—	—	—	8.3	1.5	1.3	—	—	**9.1**	**2.4**	**1.1**	**1.0**	**4.5**
A1002	—	—	1.1	1.4	5.8	5.0	—	0.9	—	—	1.8	—	—	—	—	1.5	1.6	1.8
A1301	—	—	—	1.3	—	—	—	—	—	—	25.7	—	—	—	—	—	—	—
A103	—	—	—	—	6.3	2.2	1.5	8.9	—	2.2	20.1	—	3.0	—	—	2.3	2.5	1.1
C1701	—	—	1.9	—	6.1	—	1.0	—	—	4.0	11.7	—	—	—	2.3	—	—	12.1
N501	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
O103	—	—	—	1.1	—	—	—	—	—	—	2.0	1.7	—	—	—	—	1.3	—
O303	—	—	1.9	1.6	5.6	13.6	1.4	—	—	6.3	—	1.0	3.2	2.2	2.5	—	—	4.7

[a]Peptide nomenclature corresponds to sequences shown in Table I and analysed in Figure 1.
[b]Peptide reactivity was tested by ELISA and expressed as ratio of sample/cut-off optical densities.
[c]Bold results indicate reactivity with autologous peptides.

Thirteen sera from random blood donors were also tested in each microtitre plate as negative controls. For detection of antibodies to each peptide by enzyme-linked immunosorbent assay (ELISA), a cutoff value was calculated as the mean absorbance plus four times the SD of the negative controls. Data were expressed as the ratio between the sample absorbance and the cutoff value (S/CO).

The reactivity of autologous COOH- and NH2-terminal peptides was tested with sera from the eight patients whose HVR1 region was sequenced. The cross-reactivity of these homologous peptides were also tested with sera from the same eight patients and an additional group of 15, clinically well characterised, patients part of a previously described cohort [Wong et al., 1996]. On the basis of liver biopsy evaluation, seven of these patients had a fibrosis index at 0 or 1, more than 20 years after the assumed date of HCV infection. The other eight patients, matched for the approximate time of HCV infection, age, and sex, had severe liver disease and a fibrosis index of 5, indicating liver cirrhosis.

Statistical Analysis

The difference of antibody reactivity between N- and C-terminal peptides was tested by chi square test. Mann-Whitney U-test was used to compare the difference in C-terminal HVR1 peptides expressed as S/CO values between cirrhotic and noncirrhotic patients. The level of significant difference was defined as probability less than 0.01.

RESULTS

The number of HCV variants detected by sequencing did not differ markedly between patients 1 to 4 (range, 2–6; mean, 4) who had progressed to cirrhosis, and patients 5 to 8 (range, 2–10; mean, 5) who had not.

The unrooted phylogenetic tree including all the E1–E2 partial sequences from all patients is shown in Figure 1. Substantial clustering of the sequences from patients 2, 4, and 6 is apparent. Genomic diversity of HCV from the cirrhotic patients appears restricted, as previously suggested by the small number of mutations and the high ratio of silent to replacement mutations in such patients [Lawal et al., 1997]. This restriction is especially marked in patients 1 and 3 and contrasts with the great diversity of HCV sequences in noncirrhotic subjects such as patients 6 and 8. Consequently, the number of amino acid changes in the 15- or 16-residue peptides corresponding to the immunogenic portion of HVR1 ranged from 0 to 2 and from 5 to 11 in the cirrhotic and noncirrhotic patients, respectively (Table I). The apparent discrepancies between the phylogenetic tree and the amino acid sequences shown in Table I are due to the higher frequency of silent nucleotide mutations in patients 1 to 4 and to mutations occurring outside of the HVR1 epitope region in the 158-nt sequence analysed phylogenetically.

The immune reactivity to autologous C-terminal HVR1 peptides is shown in Table II. Antibodies to one autologous peptide were not detected in each of patients 2 and 4 in the cirrhotic group. With the exception of peptide 3-1 from patient 3, serum from patients 1 to

TABLE III. Antibody Reactivity Against N-Terminal HVR1 Peptides in Patients With and Without Cirrhosis

Peptides[a] HCV sera	1-1	1-2	2-1	2-3	3-1	3-2	4-1	5-1	5-4	6-1	6-2	7-1	7-2	8-1	8-2	8-3	8-4	8-5
Cirrhotic		*				*					*							**
No1	—[b]		—	—	—		—	—	—	—		—	—	—	—	—	—	
No2	**—**		**—**	**—**	—		—	—	—	—		—	—	—	—	—	—	
No3	—		—	—	**—**		—	1.0	—	—		—	—	—	—	—	—	
No4	—		—	—	—		**—**	—	—	—		—	—	—	—	—	—	
B304	—		—	—	—		—	—	—	—		—	—	—	—	5.6	7.6	
D202	—		—	—	—		—	—	—	—		—	—	—	—	—	—	
J403	—		—	—	—		—	—	—	—		—	—	—	—	—	—	
L305	1.3		—	—	—		—	—	—	—		1.6	—	—	—	—	—	
M1003	2.9		—	—	—		1.7	—	—	3.1		—	—	—	—	—	—	
M2501	1.9		—	—	—		4.5	—	—	—		—	—	—	—	—	—	
M606	—		—	—	—		—	—	—	—		—	—	1.0	—	—	—	
W801	—		—	—	—		—	—	—	—		—	—	—	—	—	—	
Non-cirrhotic																		
No5	—		—	—	—		—	**—**	**—**	—		—	—	—	—	—	—	
No6	—		—	—	—		—	—	—	**—**		—	—	—	—	—	—	
No7	3.7		—	—	—		3.9	—	—	—		**—**	**—**	—	—	—	—	
No8	—		—	—	—		—	—	—	—		—	—	**—**	**—**	**—**	**—**	
A1002	—		—	—	—		—	0.9	—	—		—	—	—	—	—	—	
A1301	—		—	—	—		—	0.9	2.5	—		—	—	—	—	—	—	
A103	—		—	—	—		1.7	—	—	—		—	—	—	—	—	—	
C1701	—		—	—	—		—	—	—	—		—	—	—	—	—	—	
N501	0.9		—	—	—		—	—	—	—		—	—	—	—	—	—	
O103	0.9		—	—	—		—	—	—	—		1.1	—	—	—	—	—	
O303	—		—	—	—		4.5	14.4	—	1.2		7.1	—	—	—	—	—	

[a]Peptide nomenclature corresponds to sequences shown in Table I and analysed in Figure 1.
[b]Peptide reactivity was as in Table 2[b]; —, no reactivity, bold results correspond to reactivity to autologous HVR1 peptides.
*. N-terminal sequences of HCV quasispecies in this patient was identical. **. N-terminal sequence was identical to peptide 8-1.

4 had low reactivity with autologous peptides. The mean (SD) sample-to-cutoff ratio for patients 1 to 4 was 2.2 ± 2.1. In the noncirrhotic group, serum samples from patients 5 to 8 reacted with all autologous peptides, although to various extents (sample-to-cutoff ratios of 1.0 to 14.7; mean ± SD, 5.7 ± 5.1). However, the difference in autologous reactivity between the cirrhotic and the noncirrhotic group did not reach significance (two-tailed t test, $P = 0.06$). None of the patient sera, whether cirrhotic or noncirrhotic, reacted with autologous N-terminal HVR1 peptides (Table III).

Cross-reactivity of C- and N-terminal peptides from the initial eight patients was tested with sera from these eight patients and an additional 15 patients stratified according to the severity of their liver disease (with or without cirrhosis), (Tables II and III). As shown in Table IV, cross-reactivity with C-terminal HVR1 peptides (37.3%) was significantly more frequent than cross-reactivity with HVR1 N-terminal peptides (7.7%) ($P < 0.0001$) but no significant difference in the level of cross-reactivity was observed. All peptides except peptide 1-1 cross-reacted with at least one patient serum; on average, 9 of the 22 eligible patients tested cross-reacted with each peptide (range 0–13). Peptide 1-2 was recognised by 6 sera from 11 eligible cirrhotic patients and only one serum from noncirrhotic patients. Conversely, peptide 6-2 cross-reacted with 6 sera from 10 eligible noncirhotic patients and only one of 12 cirrhotic patients. Peptide 3-1 was recognised by 12/22 sera, all at high level (sample/cut-off range 2.7–8.3). Whether with N- or C-terminal HVR1 peptides, no difference in frequency of cross-reactivity was observed between sera from cirrhotic or noncirrhotic patients. On average, sera from patients with cirrhosis cross-reacted with 6.6 of the 18 C-terminal HVR1 peptides (range 0–13) and patients without cirrhosis with 6.3/18 C-terminal peptides (range 0–11). However, the mean level of cross-reactivity with the C-terminal peptides in patients without cirrhosis (mean S/CO = 5.2) is significantly higher than in patients with cirrhosis (mean S/CO = 3.1) ($P<0.001$). Since patients 7 and 8 had an inhibitor to Factor VIII, liver biopsy was not performed and we did not have direct evidence of absence of cirrhosis. We therefore conducted a comparative analysis of cirrhotic and noncirrhotic patients after exclusion of these two patients. The percentages of reactivity with the N- and C-terminus peptide did not change significantly. The mean level of cross-reactivity remained significantly higher in patients without cirrhosis ($P< 0.001$).

DISCUSSION

The data suggests that the genomic diversity of HCV quasispecies, expressed either in terms of overall nucleotide sequence, or the ratio of silent to replacement mutations is greater in noncirrhotic than in cirrhotic HCV carriers. However, the number of HCV variants did not differ between the two patient groups. These results thus differ from previous data based on SSCP or sequence analysis [Enomoto et al., 1994; Honda et al., 1994; Kurosaki et al., 1994; Sekiya et al., 1994; Moribe et al. 1995; Koizumi et al. 1995; Wilson et

TABLE IV. Antibody Cross-Reactivity of HCV Infected Patients With N- and C-Terminal Peptides

	14 N-terminal peptides		18 C-terminal peptides	
	Positive and borderline[a]	Negative	Positive and borderline	Negative
Cirrhotic	11 (6.7%[c])	152	79 (37.8%)	130
Mean S/CO[b]	2.9		3.1	
SD	2.1		4.4	
Noncirrhotic	11 (8.9%)	112	58 (36.7%)	100
Mean S/CO	3.4		5.2	
SD	3.8		5.4	
Total	22 (7.7%)	264	137 (37.3%)	230
Mean S/CO	3.1		4.0	
SD	3.2		5.0	

[a]Positive are > cut-off; borderline have S/CO >0.9 <1.0.
[b]Cut-off (CO) is determined as the mean of 13 negative controls plus 4 standard deviations. S/CO, sample to cut-off optical density.
[c]Percentage of positive result from the total number of peptide/serum combinations.

al., 1995; Toyoda et al. 1996]. Although genomic diversity was not related to duration of infection in these studies, there was a trend toward such a relation in one of these studies for which data from individual patients was available [Honda et al., 1994]. Several reasons might explain such a discrepancy. Because SSCP requires that two molecules differ by 10% of their component nucleotides to be recognised as distinct, it may underestimate the number of variants, unless each band is sequenced individually [Enomoto et al., 1994; Kurosaki et al., 1994]. However, others have argued that sequencing of random cDNA clones might miss important variants unless the number of clones sequenced is high [Honda et al. 1994; Moribe et al., 1995; Farci et al., 1996]. These researchers thus studied genomic diversity in terms of the number of mutation sites, which provides information on the rate of mutations but does not necessarily reflect the immunologically relevant changes in amino acid sequence. We showed previously that the silent/replacement mutation ratio was higher in liver transplant patients than in untreated patients and that mutations in the latter group were predominantly of the replacement type [Lawal et al., 1997]. Similarly, in the present study, cirrhotic patients showed a markedly lower number of amino acid replacements in the immunogenic portion of HVR1 than did noncirrhotic patients, despite a relatively large genomic diversity as revealed by the phylogenetic tree (Fig. 1).

The reactivity of serum from cirrhotic HCV carriers with HVR1 autologous C-terminal peptides was less than that of serum from non-cirrhotic patients, but the difference was not significant. No reactivity with autologous N-terminal HVR1 peptides was observed. This result was similar to the findings of Zibert et al. [1997] in patients infected with the same strain of HCV who became infected chronically. In this group of patients, with this particular virus, high level of reactivity with HVR1 N-terminal peptides appears correlated with recovery from HCV infection. In contrast, reactivity with the C-terminal HVR1 peptides was higher in patients who became chronic HCV carrier and this reactivity persisted over time.

Previous results [Zibert et al., 1995; Scarcelli et al. 1995; Jackson et al., 1997] indicated that C-terminal peptides cross-reacted with sera from patients infected with unrelated viral strains. We therefore tested HVR1 peptides derived from the nucleotide sequences of our patients with a panel of sera from 23 HCV chronic carriers in order to assess whether or not reactivity with either C- or N-terminal HVR1 peptides was correlated, and possibly predictive, of clinical outcome. As shown in Tables II and IV, 39% of all combinations of C-terminal HVR1 peptides and sera from HCV chronic carriers were positive for cross-reactivity tested with the peptide ELISA. The frequency of cross-reactivity did not distinguish between patients with or without severe liver disease. However, patients with mild liver disease (noncirrhotic) had a significantly higher level of cross-reactivity. A higher level of recognition of HVR1 C-terminal epitopes may reflect a stronger immune response to these epitopes of patients who do not develop significant liver disease over several decades post HCV infection. This observation is compatible with our previous findings suggesting that patients with an immune system impaired by therapeutic immunosuppression tend to have less HCV genomic diversity. Our results are similar to the cross-reactivity observed by Zibert et al. [1997] with HVR1 C-terminal peptides derived from HCV strain AD78 tested in patients chronically infected with unrelated viruses.

In contrast, N-terminal HVR1 peptides, whether autologous or homologous, had little or no reactivity with sera from patients with or without severe liver disease. This observation confirms previous data indicating that HVR1 N-terminal peptide reactivity was found during the early phase of HCV infection, particularly in patients who recovered from the infection [Zibert et al., 1997]. Chronically infected patients reacted poorly to these epitopes. However, in sera from patients who had or did not have severe liver disease, a 8% rate of reactivity was found with a mean level of reactivity approximately 3 times the cut-off, indicating that a proportion of chronically infected patients recognise unrelated HVR1 N-terminus linear epitopes, irrespective of their clinical outcome.

In conclusion, significant differences were not found in reactivity or cross-reactivity with either N- or C-terminal HVR1 peptides between HCV chronic carriers who did or did not progress to cirrhosis over a period of

more than 20 years. However, there was a significantly higher level of cross-reactivity with unrelated HVR1 C-terminal peptides in non-cirrhotic patients suggesting a possible clinical relevance of anti-HVR1 immune response in HCV infection clinical outcome.

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